gembloux agro bio tech

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Biological control formulations incorporating essential oil components

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Definitions

Biological control of insect pests :

« the use of living organisms (insects or pathogens) to suppress pest populations, making them less damaging that they would otherwise be »

(Stoner K., University of Maine, Coop. Ext. Bull., 2004)

➔ *Semiochemicals*

Definitions

Semiochemicals : in Greek « semeion » = signal

« Chemicals emitted by living organisms (plants, insects,...) that evoke a behavioural or a physiological response in other individuals »

Semiochemicals

Pheromones

- Alarm
- Sex
- Aggregation
- Trail
- Host marking
- …

Allelochemicals

- Allomones : + emitting species
- Kairomones : + receptor species
- Synomones : + emitting, + receptor

A same molecule can be a pheromone and an allelochemical substance

Aphid problem

Hemiptera: Aphididae

Aphid tritrophic system

1st level

Plant

Vicia fabae (L.)

2nd level

Herbivorous pest insect

Aphid: ***Megoura viciae*** **(Buckton)**

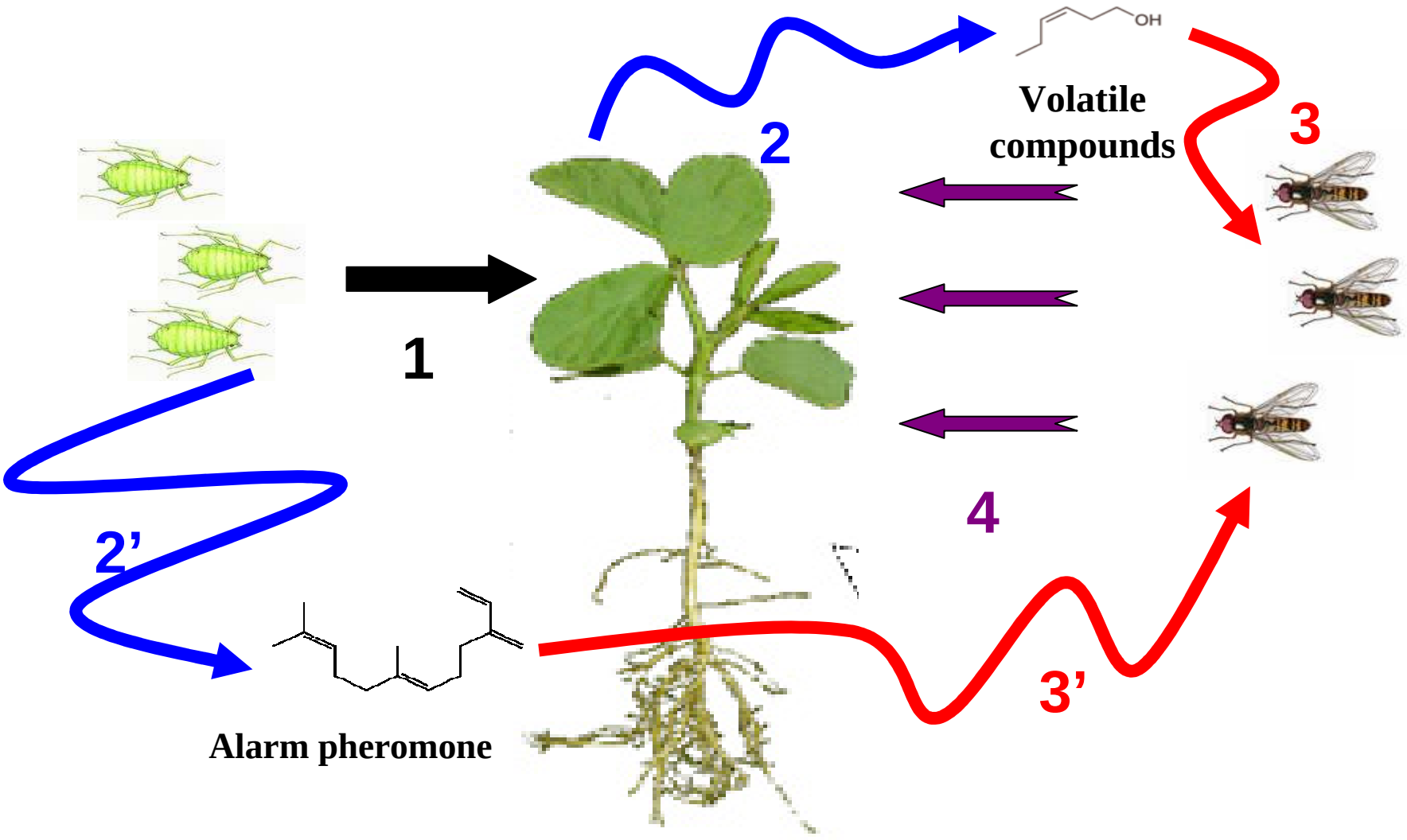
3rd level

Predator

Larva of ***Episyrphus balteatus*** **(De Geer)**

Parasitoid

Aphidius ervi **(Haliday)**


OH
Volatile
compounds
2
3
1
2’
4
3’
Alarm pheromone

E-β-Farnesene

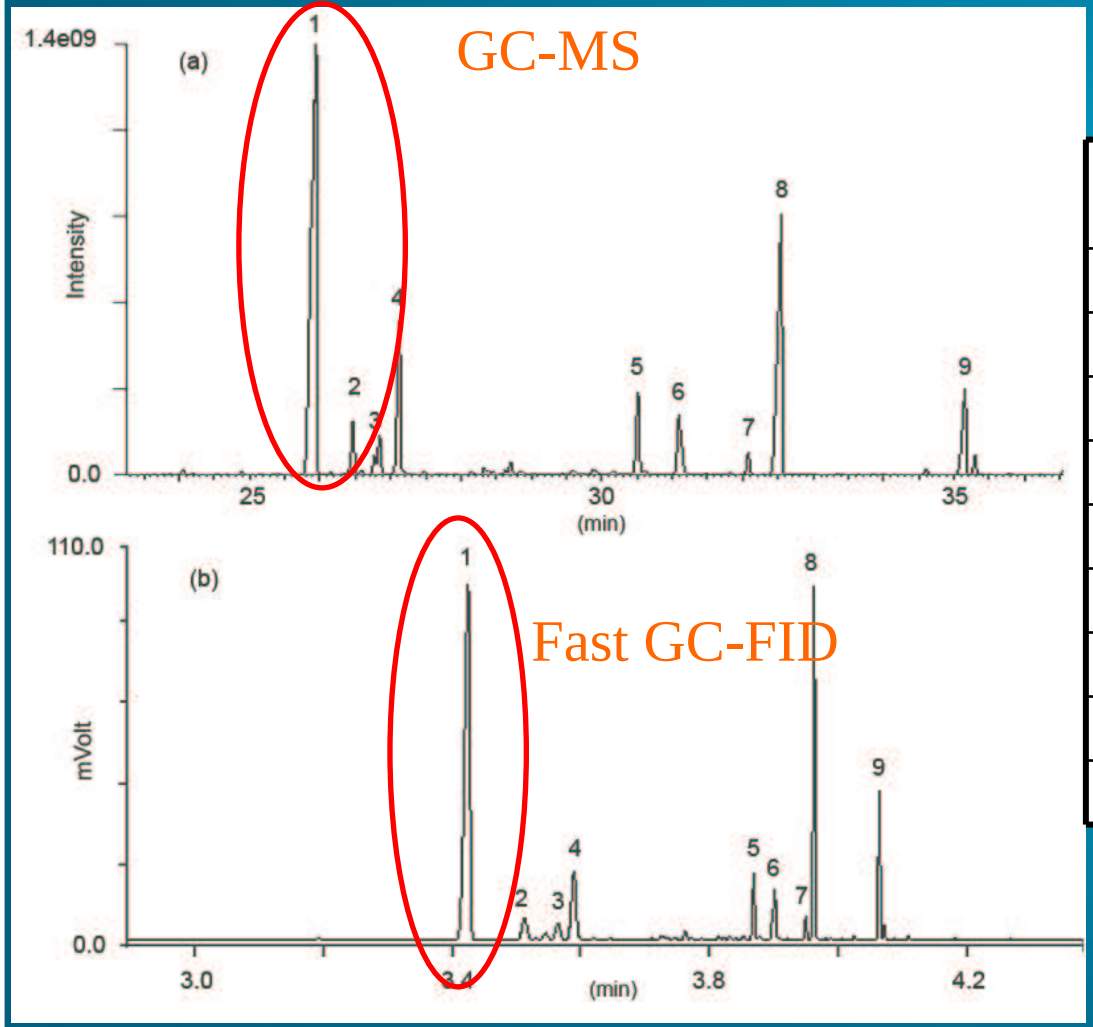
ene: $C_{15}H_{24}$

➔ Alarm phero

➔ Kairomone: ra: Syrphidae) and parasit ae) of aphids

➔ Isolated from essential oil of ***Matricaria chamomilla* L.** (Asteraceae)

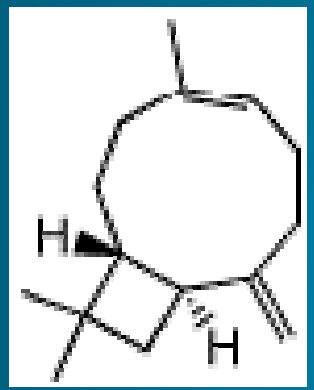
Matricaria chamomilla essential oil



Originated from Nepal (Vossen &Co., Belgium)

N°	Major compounds	Retention index	%
1	E-β-farnesene	1456	42,6
2	Germacrene D	1478	2,9
3	bicyclogermacrene	1494	1,9
4	(E,E)-α-farnesene	1506	8,3
5	α-bisabolol oxide B	1649	4,4
6	α-bisabolone oxide A	1673	4,5
7	Chamazulene	1715	1,1
8	α-bisabolol oxide A	1735	21,1
9	Cis-ene-yne-dicycloether	1802	5,9

β-Caryophyllene



Sesquite

➔Aggregation ph lady beetle,

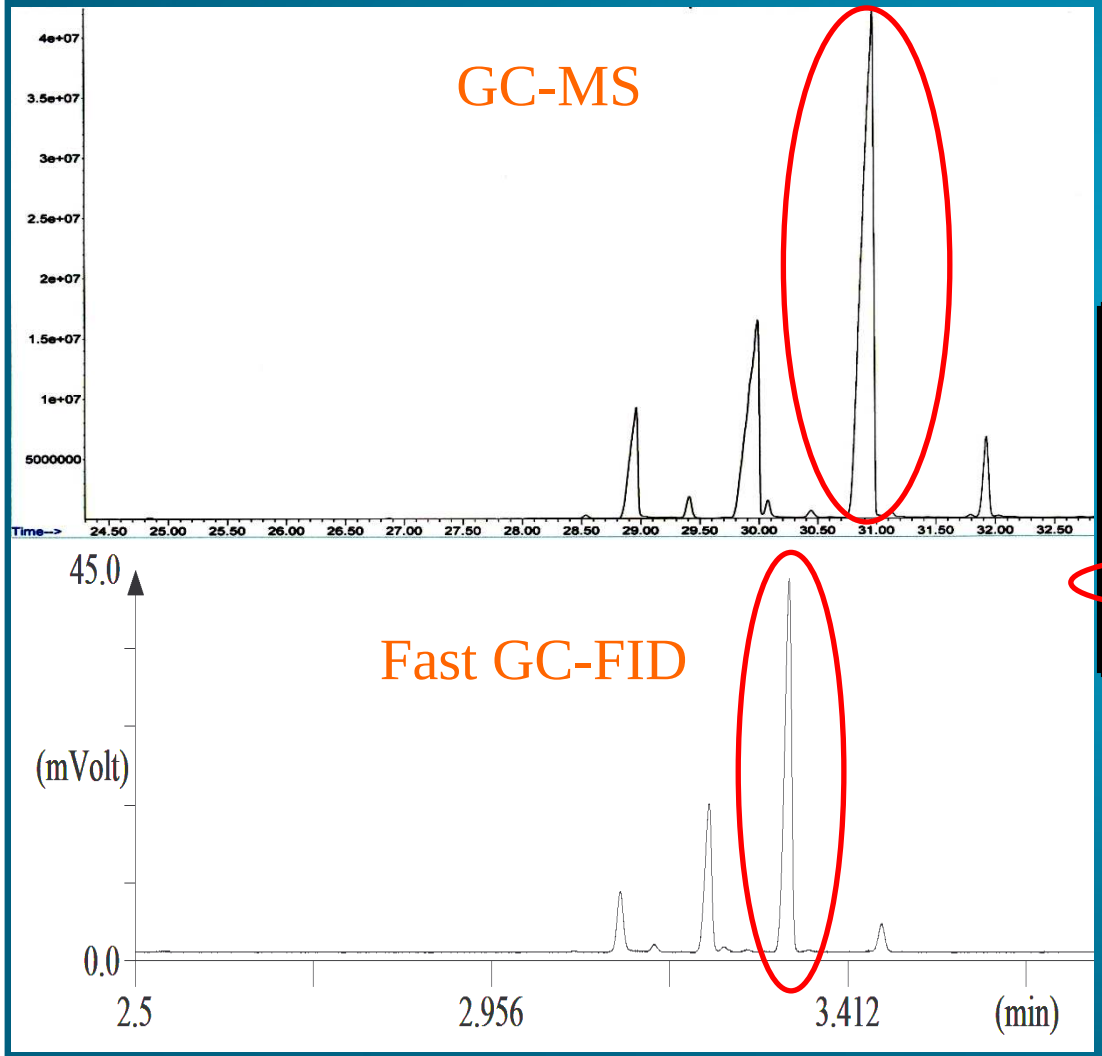
Harmonia axyridi tor)

(Verheggen F., Fagel Q., is F., Haubruge E., J. Chem. Ecol. 33 (2007) 2

➔ Isolated fro ***eta cataria* L.**

(Lamiaceae)

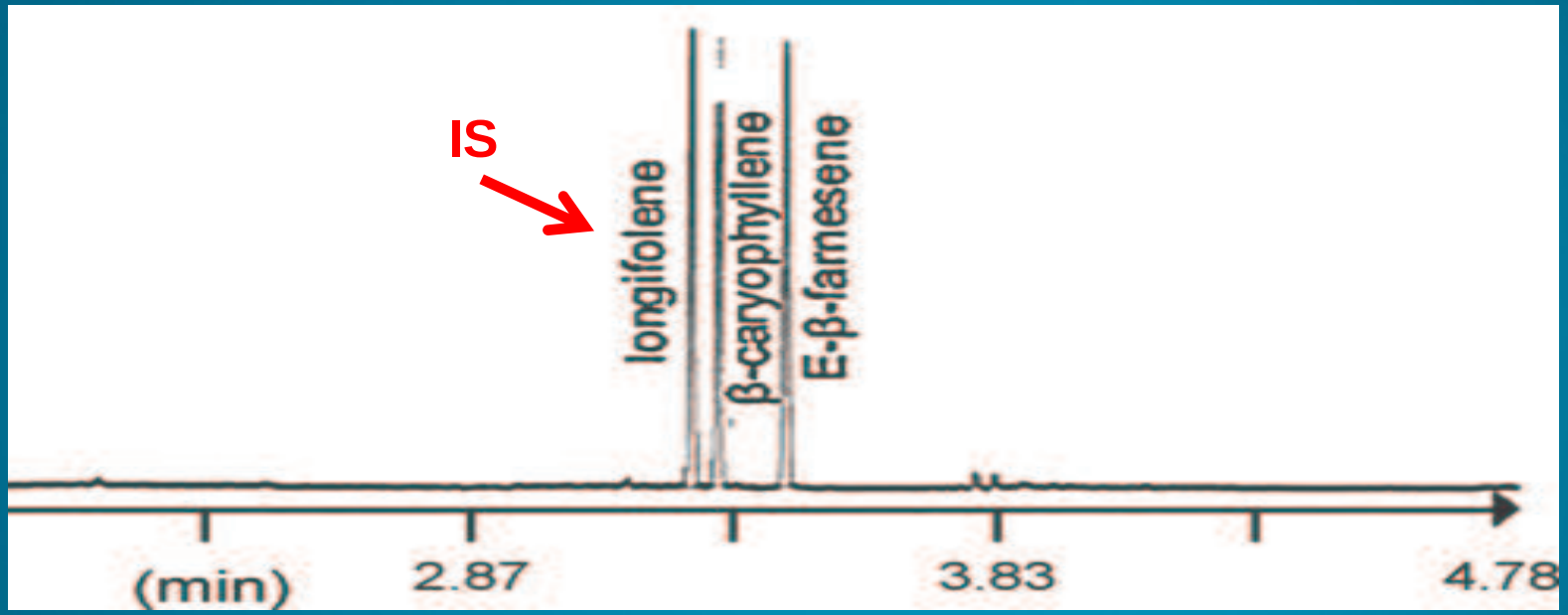
Nepeta cataria essential oil



Originated from Canada (Essential7, USA)

N°	Major compounds	Retention index	%
1	(Z,E)-nepetalactone	1353	8.4 %
2	(E,Z)-nepetalactone	1377	22.5 %
3	**β-caryophyllene**	1415	**58.9 %**
4	α-humulene	1465	3.9 %

Fast GC analytical method optimisation



Program of T° :

Initial T° : 40°C; 0.10 min

Ramp 1 : 30°C/min ➔ 95°C

Ramp 2 : 35°C/min ➔ 155°C

Ramp 3 : 200°C/min ➔ 280°C; 0.5 min

Oven run time : **4.78 min**

Ultra Fast Module : Ph5; 0.1 µm film thickness, 5m x 0.1mm I.D.

Carrier gas : He; 0.5 ml/min

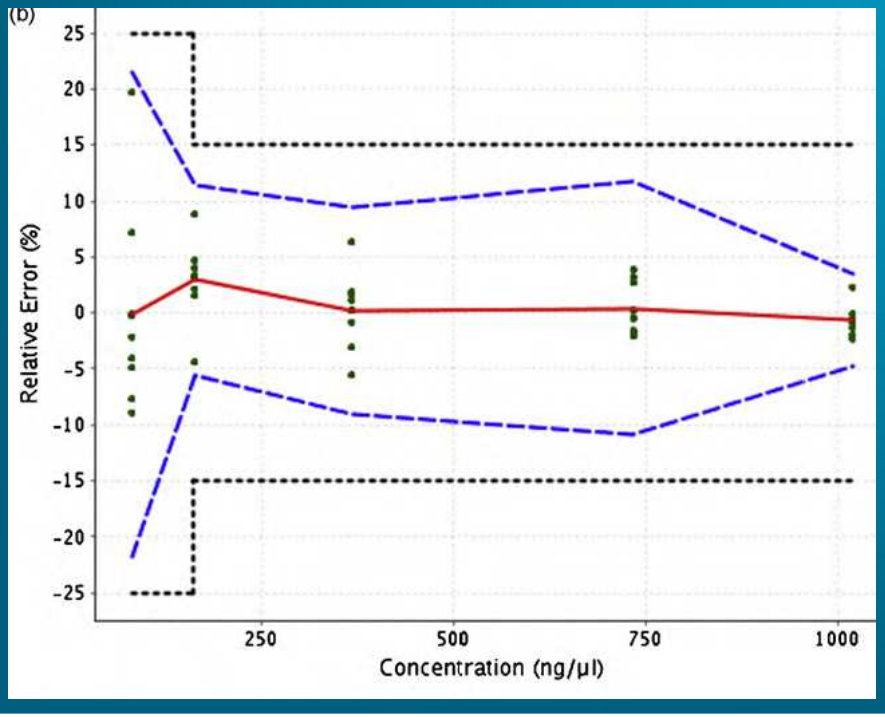
Split ratio : 1:100

(Heuskin S., Godin B., Leroy P., Capella Q., Wathelet J.-P., Verheggen F., Haubruge E., Lognay G., J. Chrom. A, 1216(14) (2009) 2768-2775)

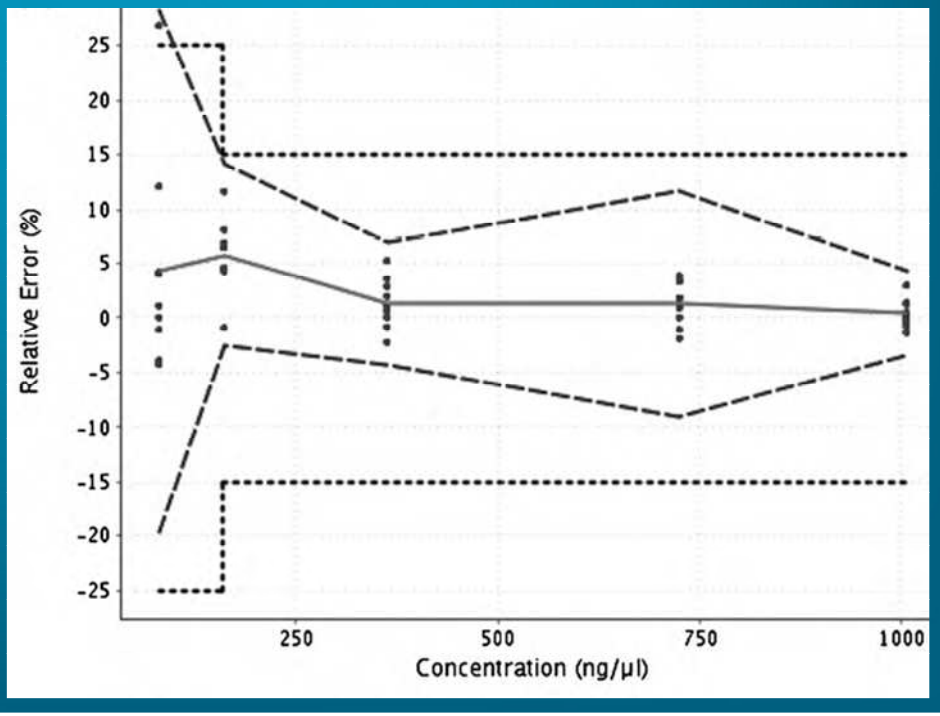
Analytical validation
Accuracy profile concept

According to the guidelines of the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP).

E-β-farnesene



β-caryophyllene



Trueness

Relative bias (%)

Systematic error

Precision

95% β-expectation tolerance limits (%)

Random error

Accuracy

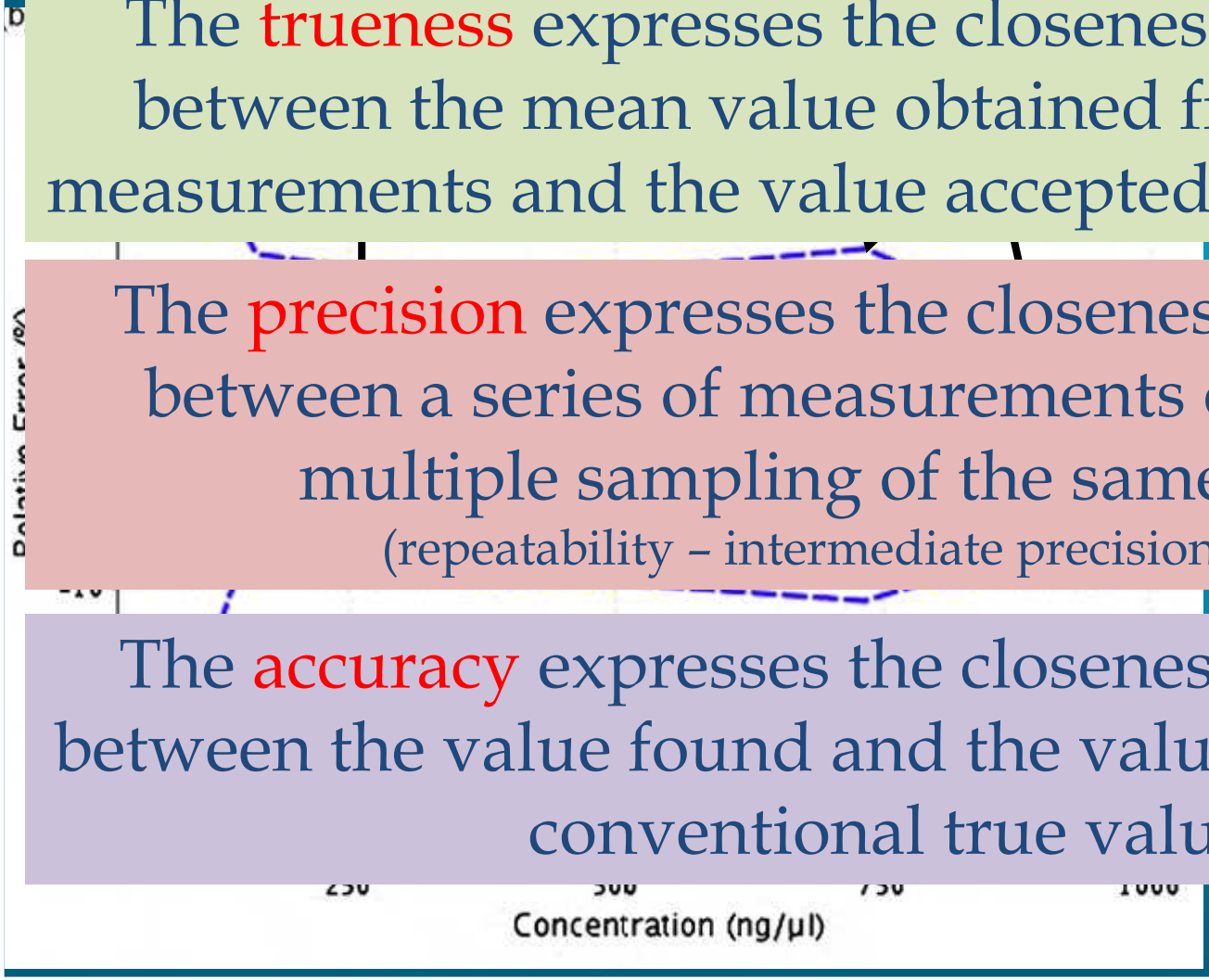
Total error

The trueness expresses the closeness of agreement between the mean value obtained from a series of measurements and the value accepted as the true value

The precision expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same sample.

(repeatability – intermediate precision (RSD%)

The accuracy expresses the closeness of agreement between the value found and the value accepted as the conventional true value



Trueness

Relative bias (%)

Systematic error

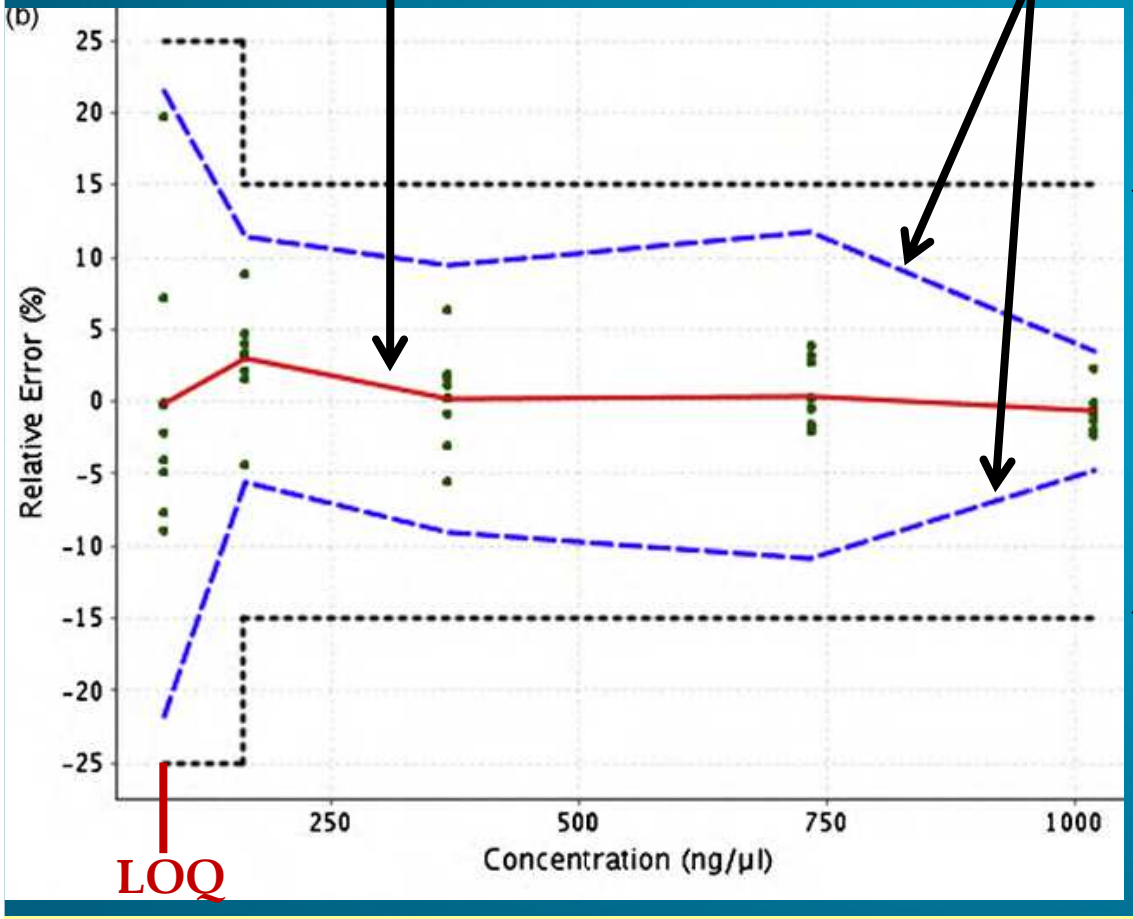
Precision

RSD (%)

Random error

Accuracy

Total error



Acceptance limits
(λ ± 15 – 25 %)

(Heuskin S., Rozet E., Lorge S., Farmakidis J., Hubert Ph., Verheggen F., Haubruge E., Wathelet J.-P., Lognay G., J. Pharma. Biomed. Anal., 53 (2010) 962-972)

Essential oil fractionation

Flash Chromatography: purification of semiochemical compounds

Composition of solvent-free semiochemical enriched fractions:

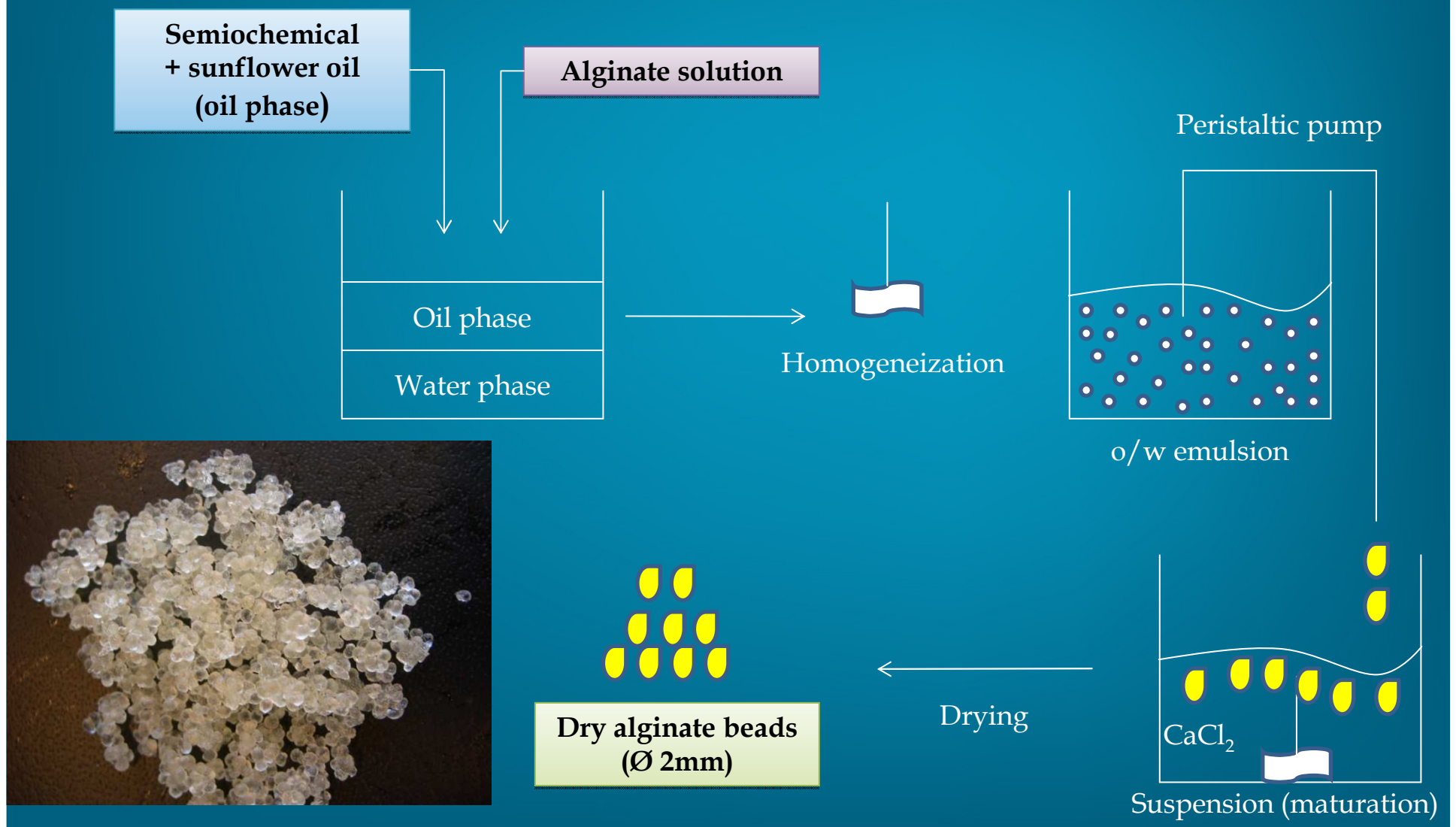
Matricaria chamomilla

Compounds	Purity
Sum of monoterpenes	1.3 %
E-β-farnesene	**84.0 %**
Germacrene D	1.4 %
Bicyclogermacrene	1.4 %
(E,E)-α-farnesene	11.9 %

Nepeta cataria

Compounds	Purity
Sum of monoterpenes	1.5 %
β-caryophyllene	**97.4 %**
α-humulene	1.1 %

Semiochemical alginate beads formulation as slow-release devices



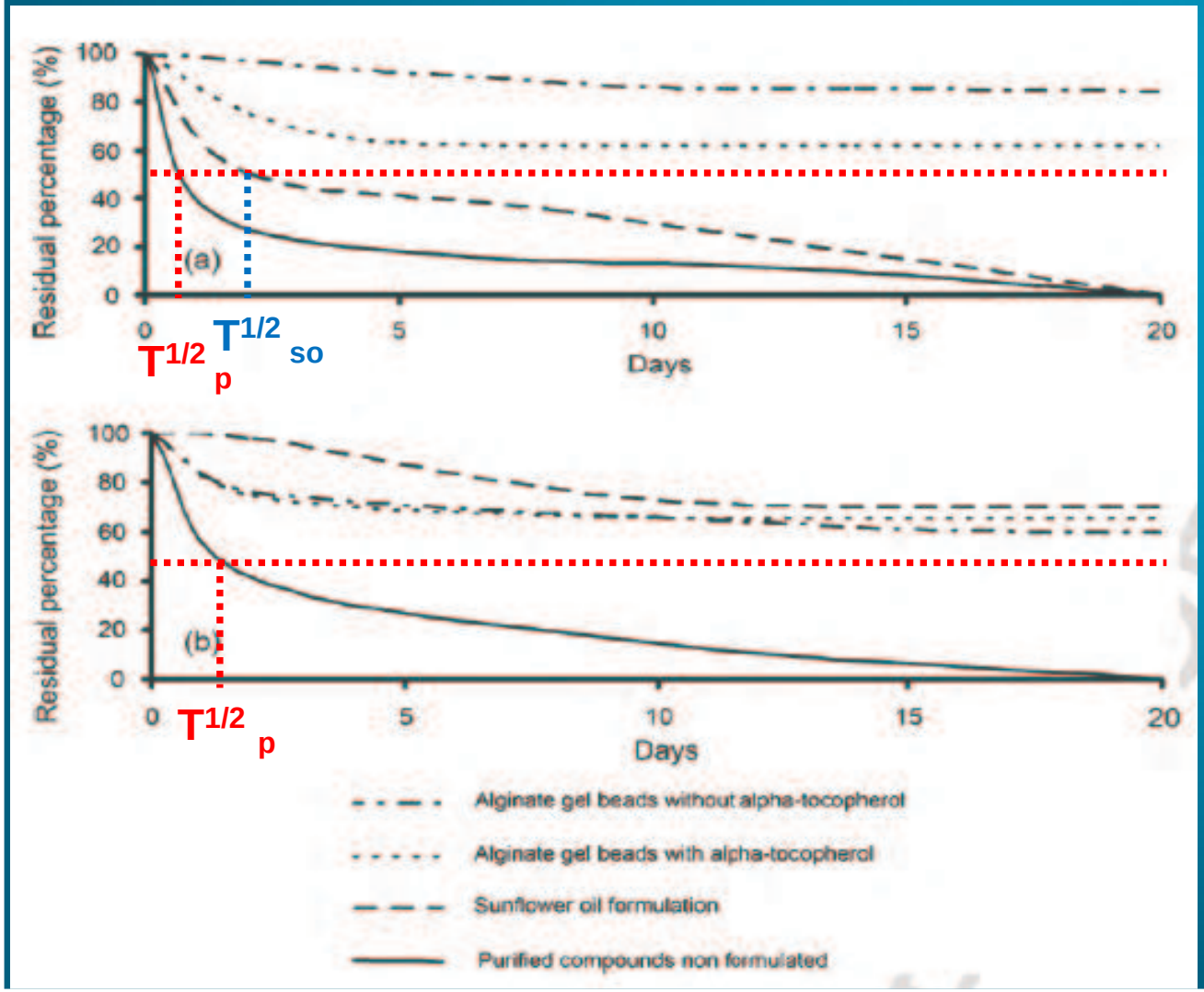
Semiochemical alginate beads formulation

➔ Optimisation of bead density and encapsulation capacity according to:

- **Alginate type: Mannuronate/Guluronate ratio**
- **Alginate concentration**
- **$CaCl_2$ concentration**
- **Reaction time in $CaCl_2$**

Semiochemical alginate beads formulation

➔ Protection efficiency of beads towards sesquiterpenes



E-β-farnesene

β-caryophyllene

(Heuskin et al., JPBA 2010)

Release rate measurement: dynamic sam

Activated charcoal filter

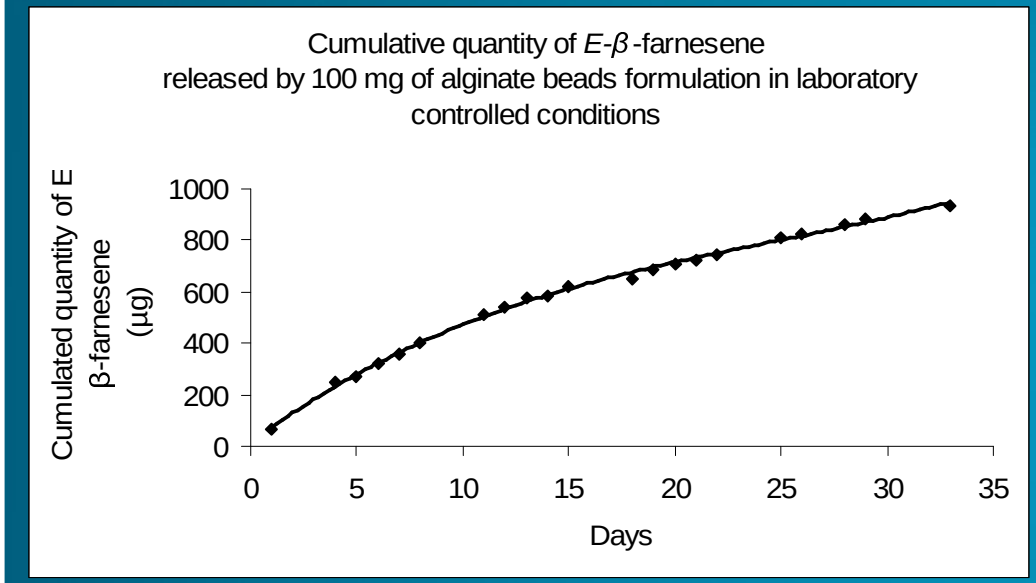
Adsorbent (SuperQ) cartridge

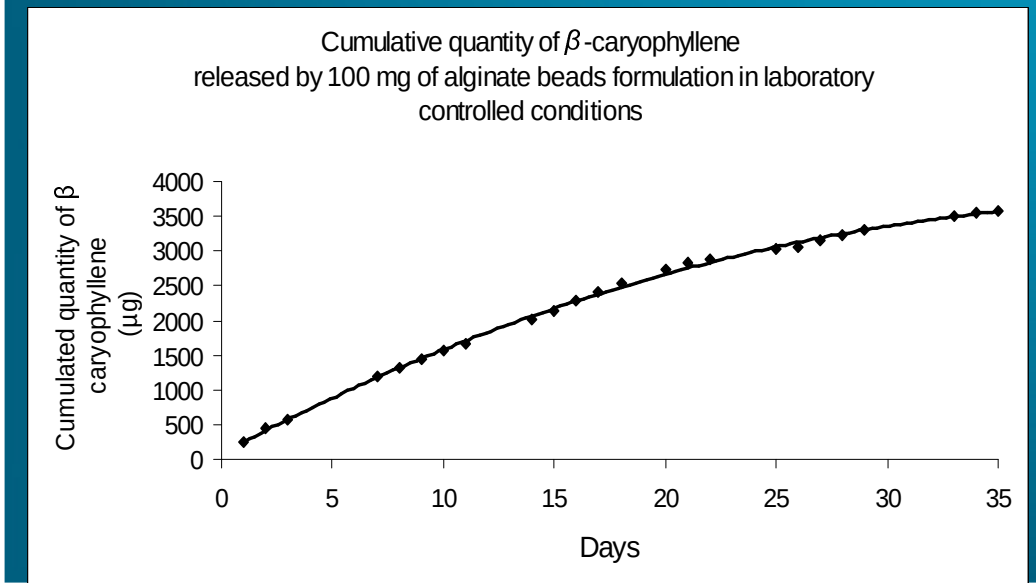
➔ ***Solvent elution + IS quantification (Fast GC)***

Teflon box with semiochemical beads

Pump

Release rate study





Laboratory-controlled conditions:

- Temperature: 20°C
- Relative humidity: 65%
- Air flow: 0.5 L/min

Release rate study

Modelisation of release rate according to physico-chemical parameters (T°, RH, wind speed)

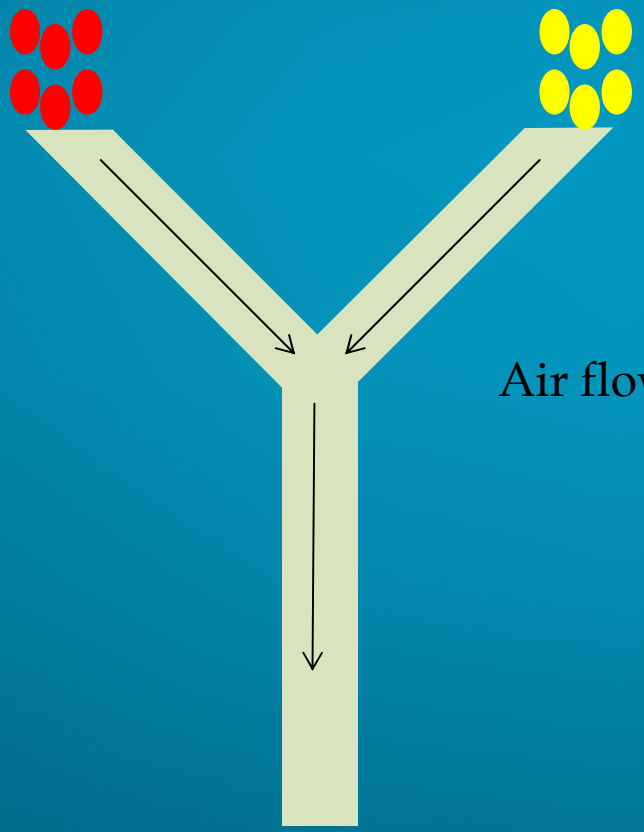
➔***Preliminary tests: diffusion coefficients estimation***

- no influence of wind speed
- influence of temperature
- influence of relative humidity

Biological tests

On parasitoids (Aphidius ervi): 2 ways olfactometer

Alginate beads without semiochemicals (blank)



Alginate beads with semiochemicals

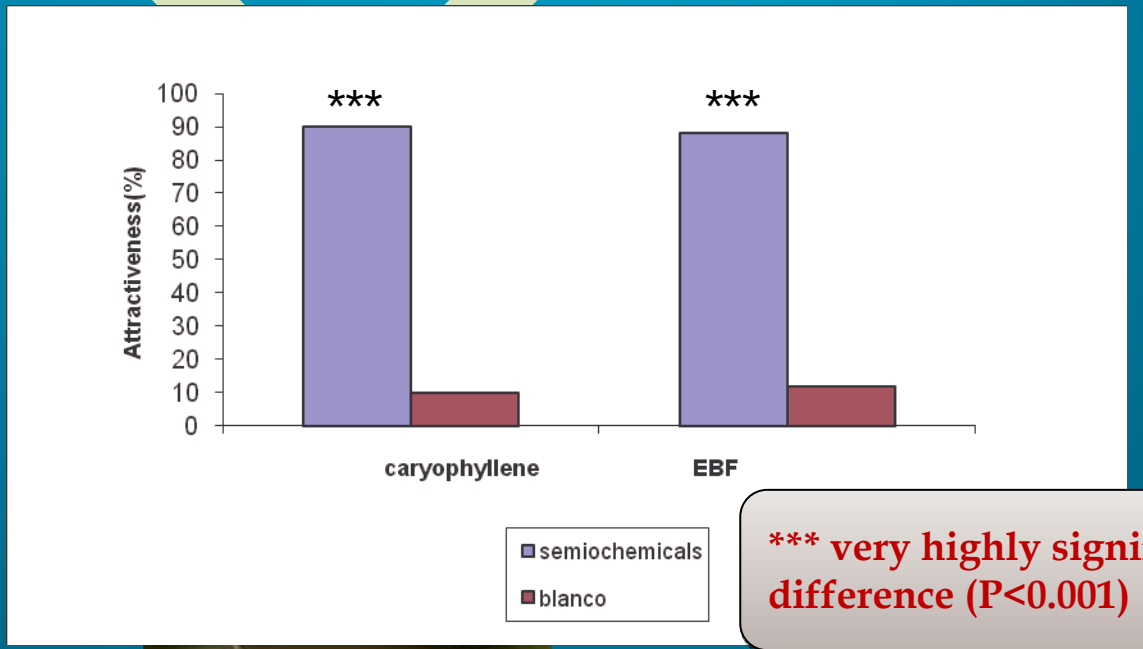
Air flow

Biological tests

On parasitoids (Aphidius ervi): 2 ways olfactometer

Alginate beads without semiochemicals (blank)

Alginate beads with semiochemicals



*** very highly significative difference (P<0.001)

Biological tests

On Syrphidae: on-field experiments (June – August 2009)

Dunnett Test (95%) : comparison of attractiveness between semiochemical beads and blank

- ***E-β-Farnesene:*** **P-value = 0.0200 (< 0.05) * significative difference**
- ***β-Caryophyllene:*** **P-value = 0.0064 (< 0.01) ** highly significative difference**

Conclusions

- Sesquiterpenes of high purity from essential oil origin
- Fast and accurate analytical method
- Semiochemical alginate beads :
  - ✓ ***efficient biological control devices***
  - ✓ ***slow-release systems***
- Slow-release modelization according to physico-chemical parameters : *In progress*

Aknowledgments

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Thank you for your attention